6th plenary days

GdR B2i

June 27 & 28, 2023
Louvain-la-Neuve
Belgique

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About GDR Bioengineering of Interfaces

The GDR Bioengineering of Interfaces (B2i) focuses on interfaces between materials and biological environment, in the broad sense. The scientific scope of this GDR includes notably the development, the characterization and the modeling of biointerfaces for applications in the fields of tissue engineering, antifouling, biosensors, etc. but also for the study of biological mechanisms or specific interactions. Because of its interdisciplinary nature, the GDR B2I federates teams active in the fields of (bio)chemistry, physico-chemistry of materials and interfaces, and biology.

The purpose of the GDR B2i plenary days is to share with the community the latest advances in research in order to stimulate new collaborations and bring out synergies between the members of the GDR. The scientific program features invited lectures, oral communications and posters as well as poster and oral awards.

Further information about the GDR B2i can be obtained on the website: https://events.femto-st.fr/GdR_B2i/fr

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Conference place

Aula Magna,
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1348 LOUVAIN-LA-NEUVE

How to get there?

Attention: do not confuse Louvain-la-Neuve and Leuven (Leuven in Flemish); the two cities are 30 km apart.

- By plane:
  - From Brussels National Airport (Zaventem), direct trains or via Brussels North to Ottignies (about 45 min) then connection (8 min) to Louvain-la-Neuve-Université.
- Brussels South Charleroi Airport serves low-cost airlines. This airport is located 40 km from Louvain-la-Neuve. The TEC bus line (line A) bring you from the airport to Charleroi Central station (20 min) where you can catch a train to Ottignies (45 min), then a connection (8 min) to Louvain-la-Neuve-Université.

- By train :

- Louvain-la-Neuve-University station is directly connected to the Ottignies railway network, on the Brussels-Namur railway line, with two trains per hour in each direction, including weekends.

- The Louvain-la-Neuve-University station is located at about 1h10 by train from the Brussels Midi station. Direct trains from Brussels South to Louvain-la-Neuve usually take longer time than taking a train from Brussels South to Ottignies and then connecting to Ottignies to Louvain-la-Neuve-Université. If you are coming from Namur or Charleroi, stop at Ottignies and then take a connection to Louvain-la-Neuve-Université.

The train schedules are available on the SNCB website.

Gala dinner: June 27, 2023 at Martin’s hotel

The gala dinner, included in the registration fee, will take place on Tuesday evening at the restaurant of the Martin's Hotel in Louvain-la-Neuve.
COMMITTEES

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UCLouvain - Institute of Condensed Matter and Nanosciences (IMCN)

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Luc Vellutini ISM, Université de Bordeaux, France
PROGRAM

JUNE 27

8:30-9:00  Registration
9:00-9:20  Welcome address (V. Humblot, K. Glinel)

Session 1 chaired by L. Vellutini

9:20-10:00  S. Bonhommeau (invited talk)  Tip-enhanced Raman spectroscopy for nanoscale chemical and structural characterization of biomolecules and biointerfaces
10:00-10:20  C. Vranckx  Layer-by-layer assembled antimicrobial nanocoatings to prevent medical implant-related infections
10:20-10:40  H. Salapare  Protein adsorption and cell response on amine- and methyl-terminated self-assembled monolayers (SAMs) on silicon
10:40-11:00  Coffee break & posters

Session 2 chaired by S. Demoustier

11:00-11:20  T. Halmagyi  Towards a highly stable, reusable and redox active gold nanoparticle-based aptasensor
11:20-11:40  F. Boulmedais  Mussel-inspired electro-crosslinking of enzymes for biosensor applications
11:40-12:00  B. Tomasetti  Solvent-free biosurface tailoring using large argon clusters
12:00-12:20  M. Lam  Meltblown-Polypropylene membrane: a material for biomedical application
12:20-14:00  Lunch & posters

Session 3 chaired by Y. Chevolot

14:00-14:40  C. Rivière (invited talk)  Agarose-based microsystems to control the mechanical and chemical environment of cells
14:40-15:00  S. Hellé  Inhibition of Pseudomonas aeruginosa by combining biosourced materials and Staphylococcus epidermidis
15:00-15:20  L. Dupont  Engineered living material based on encapsulated commensal skin bacteria
15:20-15:40  F. Barbault  Molecular modelling investigations of self-healing peptide hydrogels
15:40-16:00  Coffee break & posters
**Session 4 chaired by A. Jonas**

16:00-16:20  A. Franco  *Hydrogel Design for Corneal Drug Screening Model*

16:20-16:40  L. Ploux  *Behavioral and proteomic adaptation of microorganisms to the elasticity of biomaterial surfaces*

16:40-17:00  A. Pistol  *Polyionenes as strong tunable bactericidal polymers*

17:00-17:10  V. Schloupt  DATAPHYSICS INSTRUMENTS

17:10-17:20  S. Struyve  QUANTUM DESIGN

17:20-17:40  Posters

17:40-18:30  Free time

18:30  Cocktail + gala dinner at Martin’s hotel

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**JUNE 28**

**Session 5 chaired by S. Boujday**

9:00-9:40  S. Gabriele (invited talk)  *Sensing the curve and the spatial confinement: mechanobiology of epithelial tissues*

9:40-10:00  J. Dejeu  *Bio-Layer Interferometry (BLI): a useful tool to investigate the interactions of i-motif DNA with small molecules and proteins*

10:00-10:20  K. Graf skaia  *In-situ characterization of molecular recognition at solid-liquid interfaces by ellipsometry*

10:20-10:40  Coffee break & posters

**Session 6 chaired by V. Humblot**

10:40-11:00  S. El-Kirat-Chatel  *AFM reveals the interaction and nanoscale effects imposed by squalamine on Staphylococcus epidermidis*

11:00-11:20  S. Micciula  *Neutron reflectometry to reveal the internal structure and the interaction mechanisms of model biological interfaces*

11:20-11:40  Awards

11:40-12:00  Conclusion (V. Humblot, K. Glinel)

12:00-13:30  Lunch
Invited speakers
Sébastien Bonhommeau received his PhD in Physics of Matter at the Paul Sabatier University in Toulouse, France (2006), working on the molecular spin crossover. He performed a two-year postdoctoral appointment at the synchrotron BESSY II in Berlin, Germany (2006-2008), and a one-year stay in Toulouse as assistant professor (2008-2009). Since 2009, he has been promoted associate professor in physical chemistry with particular emphasis in spectroscopy at the University of Bordeaux (UBx), France. He was awarded a CNRS/UBx chair of excellence (2009-2014) and the instrumentation prize of the French Physical Chemistry Division of the “Société Chimique de France” (2020) for the development of tip-enhanced Raman spectroscopy (TERS). He has published nearly 50 articles in peer-reviewed international scientific journals, making important contributions in molecular magnetism and in the nanoscale chemical and structural characterization of biomolecules using TERS.
Tip-enhanced Raman spectroscopy for nanoscale chemical and structural characterization of biomolecules and biointerfaces

Sébastien BONHOMMEAU

Institut des Sciences Moléculaires, Université de Bordeaux, Bordeaux, France

Tip-enhanced Raman spectroscopy (TERS) has emerged as a powerful technique for chemical and structural characterization with nanoscale (and even sub-nanoscale) spatial resolution. TERS combines the chemical specificity of Raman spectroscopy and the high spatial lateral resolution of scanning probe microscopies (such as AFM). Several TERS configurations have been proposed for the description of biomolecules and biomaterials, either in bottom-, top- or side-illumination geometries. They allowed TERS signatures of nucleic acids, proteins/peptides, lipid membranes, viruses and cells to be unraveled.1

Here, we present scientific and technical achievements in TERS, and especially those realized at the University of Bordeaux for the characterization of biosurfaces and biointerfaces. Benefits of TERS in total internal reflection (TIR) compared with other experimental configuration will be underlined2,3 and important elements regarding the fabrication of adequate TERS probes for nanoscale chemical imaging will be discussed.4 TIR-TERS takes advantage of recent developments of surface-enhanced Raman spectroscopy (SERS) in TIR, to describe oriented biomolecules or protein assemblies on metal substrates.2 SERS studies are indeed considered as good references for the interpretation of TERS data. Our recent works in TIR-SERS and TIR-TERS will be described through the investigation of several examples (cytochrome C monolayer, amyloid fibrils and lipid membranes).2,3,5 In addition to TERS measurements in air, which are the most frequent, our activity for the development of TERS in liquid medium will be finally described. The achievement of TERS imaging in aqueous environment is the next major challenge for applications in biology.

Figure 1. Schematic representation of TIR-TERS configuration for the study of cytochrome c.2

References

Dr. Charlotte Rivière, had education in both material science engineering and biomedical engineering. After receiving a PhD in biophysics in 2005, she spent one year as an R&D engineer in a start-up company developing hybrids nanoparticles for biomedical applications (Nano-H). She then got a position of associate professor in 2006 in the biophysics group of the Institute of Light and Matter (ILM). Her research activities have covered a wide range of fields, from fundamental biophysics (mechanobiology, individual and collective cell migration) to biomedical applications (cell-biomaterial interactions, development of nano-therapies, microfluidic systems for cell biology). She now focuses on bioengineering and cancer physics and is actively involved in collaborative projects between physics, microtechnology, and cancer. Since 2021, she is hosted part of her time at the Research Center for Cancer of Lyon (CRCL), where she has just set up a new platform (uFab) to make microsystems more accessible for cancer biologists.
Agarose-based Microsystems
to control the mechanical and chemical environment of cells

Charlotte Rivière

a. University of Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, Villeurbanne, France
b. Institut Convergence Plascan, CRCL, Lyon, France
c. Institut Universitaire de France (IUF), France

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There is a number of evidence indicating that both tumor micro-environment and mechanics are playing an important role in the malignant transformation of cells and resistance to treatment [1]. We try to take into account these important issues (micro-environment and mechanics) by developing original techniques enabling precise control of the cell micro-environment, including the applied mechanical stress. In particular, we have developed agarose-based microsystems that enable precise control of the cell micro-environment in terms of mechanics (stiffness, stress) and transport of molecules (through a porous matrix) (Figure 1, [2-5]). Combined with multi-positions time-lapse microscopy and image analysis, we can decipher cell response in-situ in such situations, at the single-cell level, and over space and time. In this seminar, I will first present our agarose-based microsystems, before describing results obtained for 2D confinement or 3D models as well as how these systems can be used to assess transport and therapeutic efficacy of novel nano-therapeutics in a more physiological environment than the classical 2D in-vitro assay used. As such, it could be a valuable tool to assess the interplay between mechanics and biochemical signaling in the progression of cancer.

Figure 1. Illustration of the soft confiner (left, adapted from [1]) and of the agarose-based microsystem developed for in-toto imaging of hundreds of spheroids (middle, from [2]) and electroporation (right, from [3]).

References
(Short bio – 10 lines)

Sylvain Gabriele is Associate Professor at the University of Mons, Belgium. After a Ph.D. in polymer physics (2006), he accepted a CNRS postdoctoral position at the Aix-Marseille University (FR) to study the confined migration of leukocytes in narrow microfluidic capillaries. In 2008, he accepted a postdoctoral position at Harvard University (USA) in the Disease and Biophysics group (Prof. Kevin Kit Parker) to study the propagation of mechanical forces in neuronal networks with magnetic tweezers. He was then appointed Chargé de Recherche FNRS (2009) and joined the faculty staff of the University of Mons as an Assistant Professor to lead the Mechanobiology & Biomaterials group, working on cell mechanobiology and new biomaterials. He was elected President of the Research Institute for Biosciences (UMONS) in 2016 and was awarded in 2021 the 2016-2020 Louis Melsens Prize by the Royal Academy of Science, Letters and Fine Arts of Belgium for outstanding achievements in physico-chemistry for life sciences.
Sensing the curve and the spatial confinement: mechanobiology of epithelial tissues

Sylvain GABRIELE

Mechanobiology & Biomaterials group, Research Institute for Biosciences, University of Mons, 20, Place du Parc B-7000Mons, Belgium

The directed migration of epithelial cell collectives through coordinated movements is key to many physiological and pathological processes and is often studied at the level of large confluent monolayers. However, numerous migration processes rely on the migration of small groups of polarized epithelial clusters and their responses to external geometries. In the meantime, the development and functioning of many organisms involves the folding of epithelial monolayers that must adapt to variations of local curvature. Despite their importance on the homeostasis of epithelial systems, spatial confinement and curvature changes are difficult to reproduce, limiting our understanding of these complex mechanisms. In this presentation, we will first introduce well-defined *in vitro* systems based on micropatterned adhesive stripes to investigate the migration of small epithelial clusters with well-defined geometries. We will highlight the importance of geometry in defining the migration properties of individual cells and cell clusters, providing a conceptual framework to extract interaction rules from how active systems interact with physical boundaries. In a second part, we will introduce a photopolymerization technique using optical photomasks to form wavy hydrogels, allowing to examine how concave and convex curvatures affect the mechanical properties of epithelial monolayers and induce nuclear deformations. We will show that active cell mechanics and nuclear mechanoadaptation are key players of the mechanistic regulation of epithelia to substrate curvature.

**Figure 1.** Top (left) and side (right) confocal views of wavy epithelial monolayers cultivated on corrugated hydrogels and immunostained for actin (green), DNA (blue) and cadherin (red). The scale bar is 10 µm.

**References**

Layer-by-Layer Assembled Antimicrobial Nanocoatings to Prevent Medical Implant-Related Infections

Cédric Vranckx¹, Olivier Cornu², Christine Dupont-Gillain¹

¹ Institute of Condensed Matter and Nanosciences, Bio- and Soft Matter, Université catholique de Louvain, Louvain-la-Neuve, Belgium
² Neuro-Musculo-Skeletal Pole, Experimental and Clinical Research Institute, Université catholique de Louvain, Woluwé, Belgium

The last decades have seen an increase in the number of antimicrobial-resistant bacteria, and a lack in the development of new therapeutic options to deal with it, which could have catastrophic consequences. The World Health Organization has declared antimicrobial resistance as one of the biggest threats to humanity. Moreover, nosocomial diseases are linked, in more than 50% of cases, to the presence of a medical device (catheters, implants, etc.). To address this issue, more and more studies are being carried out to improve the biomaterial surface properties through the design of antimicrobial coatings.

Among these strategies, the use of antimicrobial peptides (AMPs) such as LL-37 constitutes an interesting avenue. These peptides are broad spectrum antimicrobial compounds which demonstrate potential as novel therapeutic agents. In order to achieve a bactericidal effect on Gram-positive and negative bacteria, the minimal inhibitory concentration (MIC) values were reported from 1 to 10 µM of LL-37. However, controlling peptide immobilization and its subsequent release or appropriate exposure is largely recognized as challenging. In order to create nanostructured antibacterial coatings, the layer-by-layer (LbL) assembly method can be used, with the benefit that the peptide is not submitted to covalent binding to the substrate, but with limitations linked to its polyelectrolyte nature and anisotropic charge distribution.

To favor its assembly, LL-37 was complexed with heparin, a polyanion, to obtain a negatively-charged peptide-polyelectrolyte complex (PPC). These PPCs were incorporated into multilayers using chitosan as polycation. As a matter of comparison, bare LL-37 was directly assembled into thin films with heparin, i.e., using a more classical LbL assembly approach. Multilayer growth was monitored using quartz-crystal microbalance (QCM), and LL-37 immobilized in the multilayer was quantified by bicinchoninic acid assay (BCA). This work aims first at studying the architecture and organization of LL-37-based multilayered films. Second, it also targets the quantification of LL-37, heparin and chitosan in the obtained films to determine whether the amount of LL-37 is sufficient to ensure antibacterial properties.

QCM results show a better growth of multilayers incorporating PPCs than those with bare LL-37. After the deposition of five bilayers, the total adsorbed mass is indeed almost three times higher with PPCs compared to bare LL-37. However, this hydrated total mass does not disclose the amount of LL-37 in the film. BCA assays show that, after the adsorption of 25 bilayers, the quantity of LL-37 is actually much higher when bare LL-37 is assembled rather than PPCs. This shows that the PPCs film architecture is completely different from the one obtained with bare LL-37. The PPCs-based multilayers are more hydrated but contain less LL-37. However, in both cases, the amount of LL-37 in the multilayers is in principle sufficient to reach the CMI.

Very importantly, our approach establishes a way to design antimicrobial coatings with different architectures and organizations. It will therefore be beneficial to modify interfaces with antimicrobial peptides in order to find new strategies against the increasing number of antimicrobial-resistant bacteria.

References
Protein adsorption and cell response on amine- and methyl-terminated self-assembled monolayers (SAMs) on silicon

Hernando S. SALAPARE III\textsuperscript{1,4}, Ricardo RAMOS\textsuperscript{1}, Arnaud PONCHE\textsuperscript{1}, Tatiana PETITHORY\textsuperscript{1}, Laurent PIEUCHOT\textsuperscript{1}, Susanne SEEMANN\textsuperscript{2}, Susanne STAETHLKE\textsuperscript{2}, J. Barbara NEBE\textsuperscript{2,3}, and Karine ANSELME\textsuperscript{1}

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The adsorption of proteins and the response of cells on the surface of materials used for implants can be understood by analyzing the physico-chemical properties of these surfaces. This understanding could allow us to develop innovative bioactive materials that addresses the increasing cases of bone deficiencies due to pathologies such as osteoporosis in developed countries [1-2]. In this study, we observed the adsorption of bovine serum albumin (BSA) protein and the spreading and migration of MG-63 osteoblastic cells on silicon substrates with varying the amine and methyl contents on the surface.

The surfaces were prepared by fabricating self-assembled monolayers with different and methyl densities on 1 x 1 cm silicon wafer substrates using a protocol that involves silanization using bromine-terminated silane, S\textsubscript{2} substitution of bromine to azide, and reduction of azide to amine [3]. The resulting surfaces were characterized by water contact angle measurements, X-ray photoelectron spectroscopy, and ellipsometry. Protein adsorption experiment was performed by immersing the samples in aqueous solutions containing BSA, afterwards, the adsorbed BSA on the surface were desorbed using phosphate buffered saline (PBS) and sodium dodecyl sulfate (SDS). The solutions from the adsorption, washing, and desorption were then analyzed by size-exclusion high-performance liquid chromatography (SE-HPLC). For the microscopic evaluation of cell-spreading, MG-63 osteoblastic cells stained with PKH26 red fluorescent Cell Linker were cultured on the silicon surfaces and the cell spreading was measured using images taken from a LSM780 confocal microscope. Lastly, for the cell migration experiment, MG-63 cells were stained with 1/2000 SPY DNA 555 nm and 1/2000 SPY FastActin650 nm and inoculated on the surface and were observed for 16.5 hours using a LSM800 confocal microscope.

The physico-chemical characterizations confirmed the presence of self-assembled monolayers on the silicon substrates with varying densities of amine and methyl groups. The adsorbed amount of BSA proteins were higher on the amine-terminated SAMs surfaces as compared with the methyl-terminated SAMs surfaces. MG-63 cell spreading was more prominent for surfaces with higher densities of amine groups. The live cell imaging also confirms the cell spreading behavior and that these cells migrate slower on surfaces with higher densities of amine groups. The results confirm the important role of amine groups on protein adsorption and cell response.

References
Towards a highly stable, reusable and redox active gold-nanoparticle-based aptasensor

Tibor G. Halmagyi\textsuperscript{a}, Mark A. Hempius\textsuperscript{b}, Julius G. Vancso\textsuperscript{b}, Corinne Nardin\textsuperscript{a}

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\textsuperscript{b} Sustainable Polymer Chemistry and Materials Science and Technology of Polymers, University of Twente, Enschede 7522 NB, Netherlands

Biosensors utilizing the specific sensitivity of custom single-stranded DNA oligonucleotide sequences, known as aptamers, would enable the facile sensing of a wide variety of materials including bacteria, viruses, and proteins. To achieve highly stable and reusable biosensors, we have assembled a system utilizing the complementary advantages of i) aptamers as bioreceptors, ii) a redox-active poly(ferrocenylsilane) (PFS) polymer as reductant and stabilizer, and iii) gold nanoparticles as transducer elements for the sensing of thrombin as model analyte (Figure 1).

In this work, we first synthesized the PFS polyelectrolyte that we then used for the mild synthesis of gold nanoparticles (PFS-AuNPs). We achieved covalent grafting of the 5’ end of a thrombin-sensitive aptamer to the PFS-AuNPs via strain-promoted azide-alkyne cycloaddition. We characterized the grafting with attenuated total reflectance (ATR-FTIR), nuclear magnetic resonance (NMR), and with enzymatic digestion, utilizing the 5’-to-3’ RecJ exonuclease to selectively digest ungrafted aptamers. Colloidal stability of the aptamer-gold composites was characterized with dynamic light scattering (DLS), and we found that the nanoparticles remained stable over 4000 s in 1 M NaCl due to the steric stabilization effect exerted by both the aptamer and the polyelectrolyte. As the aptamer conformation changes upon addition of the target, the stabilization effect decreases, leading to aggregation of the aptamer-PFS-AuNPs, which we followed with DLS. The model system is sensitive to thrombin at concentrations ≤ 0.5 μM and was found specific in control tests against beta-lactoglobulin. Additionally, the PFS renders the system redox sensitive, as evidenced by a strong oxidant, FeCl₃, inducing aggregation of the aptamer-PFS-AuNPs, making our model redox-active aptasensor assembly multifunctional and potentially reusable.

![Figure 1. Working principle of the thrombin-specific aptamer-PFS-AuNP nanosensor.](image-url)
Mussel-inspired electro-crosslinking of enzymes for biosensor applications

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Complementary tools to classical analytical methods, enzymatic biosensors are widely applied in medical diagnosis due to their high sensitivity, potential selectivity, and their possibility of miniaturization/automation [1]. Among the different protocols of enzyme immobilization, the covalent binding and cross-linking of enzymes ensure the great stability of the developed biosensor. Obtained manually by drop-casting using a specific cross-linker, this immobilization process is not suitable for the specific functionalization of a single electrode out of a microelectrode array.

In this work, we present a mussel-inspired electro-cross-linking process that was validated using polymer bearing amino groups and used to develop an enzymatic biosensor to measure glucose. A homobifunctionalized catechol ethylene oxide spacer or tannic acid (polyphenol bearing gallol moieties) were used as cross-linker in the presence of glucose oxidase (GOX). Performed in one pot, the process is based on the chemical reaction of electro-oxidized catechol/gallol moieties with free amino moieties of GOX through Michael addition and a Schiff’s base condensation reaction [2]. Using tannic acid, cheap and abundant natural molecule, allowed the synthesis of gold nanoparticles embedded in the film leading to higher sensitivity [3].

![Figure 1. Schematic principle of Mussel-inspired electro-crosslinking of enzymes with tannic acid coated gold nanoparticles](image)

Finally, this mussel-inspired cross-linking of enzymes (i) is obtained in a one pot and versatile process, likely to be applied on any kind of enzymes, (ii) does not require the synthesis of a specific cross-linker, (iii) gives enzymatic biosensors with high and very stable sensitivity over two weeks upon storage at room temperature and (iv) is temporally and spatially controlled, allowing the specific functionalization of a single electrode out of a microelectrode array. Besides the development of microbiosensors, this process can also be used for the design of enzymatic biofuel cells

References
Solvent-free biosurface tailoring using large argon clusters

Benjamin Tomasetti

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The ability to biofunctionalize surfaces with proteins is a major challenge in many fields such as biocatalysis, tissue engineering or biomedical devices. The proof-of-concept of the transfer of fragile biomolecules from a target reservoir to a collector substrate using large Ar gas cluster ion beams \((\text{Ar}_n)^+\text{-GCIB}) was recently demonstrated in our laboratory [1]. Indeed, these nanometeories, composed of thousands of Ar atoms and accelerated to 10 keV in vacuum, have the amazing property to sputter intact molecules as large as 14 kDa.

Currently, the construction of new biomaterials such as thick multilayers has become possible for several reasons (Figure 1). First, because no solvent is used, separate layers can be built on any collector substrate (e.g. paper, PET, silicon, gold, etc.). Second, properties such as deposition thickness and bioactivity can be controlled very precisely because they are linearly proportional to the total \(\text{Ar}_n^+\) ion dose used to bombard the reservoir [2].

Finally, the molecular transfer is used to increase ToF-SIMS sensitivity for molecules in tissues. A microvolume from the tissue sample is expanded to a collector, which can be any favorable material. The goal is to increase the signal when the collector is then analyzed using a liquid metal ion gun (LMIG).

Figure 1. Schematic representation of the successive transfer from a target to a collector of two different biomolecules. A first layer is built by the transfer of the green biomolecule and a second layer is built on top by the transfer of a second blue molecule. A 3D Time-of-flight secondary ion mass spectrometry (ToF-SIMS) image of a multilayer construction is shown at top left.

References
Meltblown-Polypropylene membrane: a material for biomedical application

M. Lam1; M. Baudoin2; B. Mougin3; C. Falentin-Daudré1,*

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2 MeltBio, 56 Rte de Ferrossière, 38110 Saint-Didier-de-la-Tour, France

The melt-blown process is an innovative technic to produce high-quality nonwoven structures composed of thousands of entangled microfilaments. The principle relies on a melted state polymer that goes through tiny nozzles, then blown by hot air flow to create randomly aligned micro-stretched filaments to compose a final membrane. Using no solvents and being easy to implement makes it the preferred method for applying biomedical devices. Membrane structures are commonly used for tasks such as filtration or wound dressing. Depending on the intended use, it is important to control the pore sizes, thickness, and compacity of the membrane, and the melt-blowing process is ideal for adjusting these parameters [1].

In this work, we study polypropylene melt-blown membranes and their bioreactivity resulting from grafting a bioactive polymer on top of the surface. Despite the acceptable biocompatibility that widens the use of polypropylene, it is not exempt from drawbacks. For some reason, bacterial issues and lack of integration exemplified this statement [2].

Thus, a suitable and reliable method was developed to graft the poly (styrene sodium sulfonate) – PolyNaSS over a hydrophobic polypropylene surface to achieve new properties targeting controlled biological responses. The grafting method uses UV irradiation (365nm) to create radical species from the surface, enabling the polymer’s direct and progressive growth over the surface with a radical polymerization mechanism. The resulting surface properties were investigated using colorimetry, water contact angle measurements, and scanning electron microscopy. Differences related to the grafting rates and improved surface wettability were evidenced. The next step is looking for cell responses to these surface contact. Ultimately, outcomes enlighten enhanced cell adhesion with improved cell morphology. In addition, fibroblasts adopted an active stretched shape on a grafted surface, whereas they took a non-active round shape on bare polypropylene. These results open new perspectives in melting-blown polypropylene membrane applications.

Figure 1. Study of UV grafted polypropylene meltblown membrane bioreactivity

References
[1] https://www.texinov.com/production-francaise-de-meltblown/
Inhibition of *Pseudomonas aeruginosa* by combining biosourced materials and *Staphylococcus epidermidis*

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2 IPREM UMR5254 CNRS/UPPA Pau, France.
3 IS2M UMR7361 CNRS/UHA Mulhouse, France.

In case of diabetes or burns, skin wounds are a gateway for bacteria to infect patients. Bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*) are particularly harmful due to their facility to become resistant to therapeutic agents. In recent years, playing with skin microbiota¹² has become a hot topic, for both therapeutic and cosmetic purposes, and represent a novel opportunity way for anti-pathogen therapy. More specifically, *Staphylococcus epidermidis* (*S.epidermidis*), a commensal bacterium, can have a “probiotic” function by preventing colonization of the host by severe pathogens¹³.

We studied the antibacterial effect of *S. epidermidis* included in hydrogel containing agarose, a biosourced component, supplemented by honey, a natural extract. Depending on the formulation, the growth of *P. aeruginosa* could be fully inhibited in hydrogels composed of agarose, honey and *S. epidermidis*. In this communication, I will present the antibacterial activities of this new hydrogel and the physico-chemical and biochemical properties that may contribute to explain these results.

References

Engineered Living Material based on Encapsulated Commensal Skin Bacteria

Louise DUPONT, Alain M. JONAS, Karine GLINEL

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The skin, which is the largest human organ, is inhabited by a myriad of microorganisms known as the “skin microbiota”. Among these, commensal skin bacteria provide their hosts with benefits resulting from the products they secrete which act directly on host skin or on other microorganisms such pathogens. Nowadays it is increasingly accepted that some skin diseases are related to alterations in the composition of human skin microbiota. Therefore, the emergence of bacteriotherapy as an alternative treatment to antibiotics to treat skin disorders seems promising. However, such an application requires to mitigate the potential negative effects of commensal skin bacteria if left free to proliferate. This can be achieved through the bacteria encapsulation.\textsuperscript{[1-3]}

In this context, the objective of our research is to develop a living material based system, called a “bacterial patch” (Figure 1), directly applicable on human skin and entrapping commensal bacteria. This patch prevents bacteria from escaping and proliferating in an anarchic way, while keeping them metabolically active in order to exploit their benefits for the treatment of skin disorders.

The patch is composed of an open silicone reservoir filled with a nutritive hydrogel loaded with \textit{Staphylococcus epidermidis} and covered with a semi-permeable film. While the silicone envelope prevents the dehydration of the hydrogel, the semi-permeable film which is brought into contact with the skin allows the outward diffusion of the molecules secreted by bacteria. In this communication, we will first present the fabrication of the patch, notably the materials tested to form the semi-permeable film and the assembly of this film onto the silicone reservoir filled with hydrogel. Two different approaches based on a polycarbonate membrane or a double polyacrylamide-agarose network hydrogel have been developed to form the semi-permeable layer. Then, we will focus on the influence of the characteristics of the nutritive hydrogel on the bacterial growth. We will show how the composition and the strength of the hydrogel influence the proliferation pattern, the metabolic activity and the functionality of bacteria.

![Figure 1. Structure of the bacterial patch.](image)

References
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\textsuperscript{[3]} W. Xu et al., Adv. Mater. Interfaces 2022, 9(7), 2102261.
Molecular modelling investigations of self-healing peptide hydrogels

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Hydrogels are 3D networks either composed of crosslinked hydrophilic polymers, block-copolymer micelles, colloids, or peptides[1]. These networks are capable of swelling in water resulting in the formation of soft materials that bear similarities to biological tissues due to their high-water content, porous structure, and mechanical properties[2].

We aim to develop supramolecular hydrogels with stimuli responsive properties using sequence-defined low molecular weight hydrogelators (LMWH). Specifically, we observed the successful gelation of a tripeptide Fmoc-FpY with a tyrosine phosphate group (pY) that imparts water solubility and self-assembly properties in the presence of NaCl. Interestingly, these hydrogels bear different structural organizations according to the temperature or ionic strengths, leading to tunable hydrogels with various physical properties. To understand these effect at the atomic scale, numerous computational studies were conducted, mainly through molecular dynamics, for various environments (concentration, temperature, packing) and compared to experimental data.

![Figure 1. Left: example of a starting and final structure of peptide organization. Right: by considering their periodic images, illustration of hydrogels fibers generation.](image)

The present study reveals a high degree of agreement between the experimental observations and the theoretical models, thereby highlighting the efficacy of a computational methodology in generating meaningful outcomes. Collectively, these results underscore the prospective applicability of computational investigations in the design and advancement of supramolecular hydrogels exhibiting desirable properties.

References
Hydrogel Design for Corneal Drug Screening Model

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The cornea is a vital transparent tissue of the eye that protects it and maintains clear vision. Corneal pathologies can cause visual impairment, impacting quality of life and increasing the risk of other health issues. In reason of aging of the population, the prevalence of visual impairment, including corneal blindness, is expected to triple by 2050.\textsuperscript{1} There is significant interest in accurately determining the pharmacokinetics of new ocular drugs. However, current models, mainly based on in vitro cell models, animal models or mathematical simulations, show a tremendous discrepancy with the corneal environment, limiting their predictive power for human trials. Currently, a correct corneal microphysiological model that includes all cell layers in an organized manner is lacking in the field.\textsuperscript{2}

One promising strategy to overcome these shortcomings is the development of a cornea-on-chip. This is the goal of an ambitious interdisciplinary project spanning across multiple institutions in Belgium, France and Austria (Figure 1). The focus of my thesis is on characterizing the properties of hydrogel biomaterials mimicking the human corneal stroma. A range of techniques including ToF-SIMS, SEM, AFM, rheometry and confocal Microscopy are used to analyze the molecular composition, fiber formation, mechanical properties, and swelling behavior of the materials. First results show how different crosslinking chemistries can be used to fine tune hydrogel mechanical properties. With the cornea-on-chip ready, advanced drug diffusion studies will be performed with TOF-SIMS to compare the results with the human cornea and determine drug retention reservoirs.

\textbf{Figure 1.} Graphical abstract of the Artificial Lithographic Model for corneal drug screening project. An artificial human cornea is recreated inside a microfluidic chip device. The stroma is fabricated using a hydrogel with 2 photon polymerization bioprinting. The epithelial cell layers are recreated using cell seeding.

References


Behavioral and proteomic adaptation of microorganisms to the elasticity of biomaterial surfaces

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Intrinsic properties of the material surface can be exploited to reduce (even though not eradicate) the microbial colonization of biomaterial surfaces. They may complete the biocidal action of antimicrobials-incorporating biomaterials by reducing the quantity of adhered microorganisms and the remaining microorganisms can be treated with classical drugs. They may even replace it if the reduction of infection risk rather than the eradication is sufficient. Mechanical properties of materials are a possible way to impact biofilm formation. However, the behavioral, physiological and metabolic adaptation to such coating properties are still questioned. We will here review some recent results we obtained regarding the adaptation of one yeast (Candida albicans) and one bacteria (Escherichia coli) species to the surface viscoelasticity of biomaterials, combined or not with hydration. Adhesion, further biofilm formation, mobility and protein production will be especially considered. In general, surface stiffness actually impacts the amount, physiology, protein production and mobility, of the adhered cells, but differently according to the biomaterial’s nature. This also varies with the microbial species. This finally confirms the relevance but also shows the difficulty of playing with the softness and stiffness of surfaces to significantly reduce the infection risk on biomaterials.

Figure 1. Schematic view of the E. coli number related to the Young’s modulus of biomaterial’s surfaces.

References
Polyionenes as strong tunable bactericidal polymers

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Microbial contamination of surfaces is a major issue in numerous fields with food, medical and industrial applications. Indeed, an uncontrollable proliferation of microorganisms poses obvious short-term health concerns (infection, food-poisoning, etc.). However, as a consequence of delayed detection and eradication, it can evolve into biofilms which further improve its persistence and resilience to conventional decontamination methods [1].

The aim of our project is to develop new strategies to prevent bacterial growth, based on antimicrobial peptides-like polymers (AMP). The polymers family we investigated called polyionenes retains the AMP’s alternative structure between cationic and hydrophobic segments, in the form of quaternary ammoniums and aliphatic carbons [2]. This chemical architecture is easily synthetized in a single polymerization step, and the wide range of usable monomers allows a fine adjustment of its physical properties.

Through an exploratory study, we designed new types of polyionenes. Each of them display variations of its lipophilic properties and also insertion of chemical groups in order to obtain new biological properties. After synthesis of the new polymers, we investigated the minimal concentration of polyionene in solution that prevents bacteria development (called minimal inhibitory concentration) of two bacterial strains (E. coli & S. aureus), and their cytotoxic threshold, beyond which more than 30% of mammalian cells die.

As a first conclusion, we observed that our polymers are competitive with the literature, regarding these value of minimal inhibitory concentration and cytotoxicity. Moreover, we discovered that our polymers, with a simple insertion of an aromatic ring, can either be non- or very specific against our two Gram+ and Gram- strains.

In addition to this study, we conducted shotgun proteomics experiments to investigate the protein response of E. coli to our polyionene. We classified several functional classes of protein that were up or down modulated and identified the proteins that were the most over- or under-expressed. To our knowledge, it is the first time that the proteomic studies have been performed to characterise polyionenes microbial properties.

References
ORAL PRESENTATIONS
June 28, 2023
Bio-Layer Interferometry (BLI): a useful tool to investigate the interactions of i-motif DNA with small molecules and proteins

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i-Motifs of DNA (hereafter, i-DNA), known in vitro for nearly three decades, are unusual, four-stranded structures, in which cytosines are intercalated via a stack of hemi-protonated C=C base pairs (CH+:C) (Fig. 1A, B). Some of these structures have been well characterized in vitro and, because i-DNA may mirror other four-stranded G-rich structures (G-quadruplexes) present in gene promoters or at telomeres, their biological relevance is being investigated.

![Figure 1. A/ Hemi-protonated C-C base pair. B/ Schematic structure of the telomeric i-DNA.](image)

Relatively few molecules were reported to interact with i-DNA. The main issues in this regard are the strong pH-dependency, flexibility, polymorphism and complex folding behavior of i-DNA, which introduce potential bias into screening methods. In particular, low-pH conditions used to induce the formation of i-DNA lead to the protonation of many ligands, which can strongly increase their non-specific nucleic acid binding. This latter point is particularly critical because the use of small molecules to study the biological functions of such structures is essential.

In this context, we are developing a method using the Bio-Layer Interferometry (BLI) to screen and to study the interactions between the i-motif and already reported ligands (TMPyP4, mitoxantrone, IMC-48, berberine, etc) at physiologically relevant pH. We demonstrated that none of the reported ligands were shown to discriminate between folded and unfolded i-motif structures.[1,2]

References
In-situ characterization of molecular recognition at solid-liquid interfaces by ellipsometry

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Interfacial processes are playing an essential role in artificial and natural systems and their characterization is thus of crucial importance. Due to its non-invasiveness and high sensitivity, ellipsometry is an excellent tool for investigating processes at liquid-solid interfaces in bio-related systems. Here, the recognition process of complementary and non-complementary synthetic oligomers that have a precise sequence of hydrogen-binding groups (DNA-base analogues) is investigated by in situ ellipsometry. Using a methodology recently developed in our group, we synthesized a series of complementary and non-complementary sequence-defined and stereo-controlled tetramers based on four monomers with the following side-groups: guanine (G), cytosine (C), a 2,6-diaminopyridine derivative (D) and thymine (T). Specific hydrogen bonds can be formed between base pair analogues G–C and D–T. Compared to DNA oligomers, the oligo(urethane-triazole) synthetic backbone of the chains provides chemical stability at the expense of a higher flexibility (Figure 1). To study recognition at the solid-liquid interface, we grafted all-R probe chains with TGCT monomer sequence onto silicon wafers, which were immersed in solutions of the complementary and non-complementary all-R target chains with monomer sequences DCGD and TTTT, respectively. Recognition experiments were performed in a liquid cell, using a mixture of acetonitrile and dimethylsulfoxide ACN:DMSO (5:1 v:v) as a solvent. In addition to the ellipsometry study, the grafting density, thickness and smoothness of the probe chain layer were characterized by x-ray reflectometry and water contact angle measurements. To analyze the recognition process in detail, different spectroscopic and kinetic ellipsometry measurements were performed. The volume coverage of the oligomers was chosen as parameter describing the molecular recognition. Our results indicate that efficient recognition between complementary oligomers can be achieved by controlling the average conformation of the grafted synthetic strands by tuning their steric crowding (i.e., varying their grafting density or inserting poly(ethylene glycol) constraining chains in the grafted layer).

References
AFM reveals the interaction and nanoscale effects imposed by squalamine on Staphylococcus epidermidis

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The Gram-positive bacterium Staphylococcus epidermidis is responsible for important nosocomial infections [1]. With the continuous emergence of antibiotic-resistant strains, the search for new treatments has been amplified in the last decades. A potential candidate against multidrug-resistant bacteria is squalamine, a natural amino-sterol discovered in dogfish sharks [2]. Despite its broad-spectrum efficiency, little is known about squalamine mode of action. Here, we used atomic force microscopy (AFM) imaging to decipher the effect of squalamine on S. epidermidis morphology, revealing the peptidoglycan structure at the bacterial surface after the drug action. Single-molecule force spectroscopy with squalamine-decorated tips shows that squalamine binds to the cell surface via the spermidine motif, most likely through electrostatic interactions between the amine groups of the molecule and the negatively-charged bacterial cell wall. We demonstrated that - although spermidine is sufficient for the initial attachment of squalamine to S. epidermidis – the integrity of the molecule needs to be conserved for its antimicrobial action. A deeper analysis of the AFM force-distance signatures suggested the implication of the accumulation-associated protein (Aap), one of the main adhesins of S. epidermidis, in the initial binding of squalamine to the bacterial cell wall. This works highlights that AFM -combined with microbiological assays at the bacterial suspension scale- is a valuable approach to better understand the molecular mechanisms behind the efficiency of squalamine antibacterial activity.

Figure 1. AFM images of S. epidermidis cells before and after squalamine treatment and principle of force measurements with squalamine tips together with a representative force-distance curve.

References
Neutron reflectometry to reveal the internal structure and the interaction mechanisms of model biological interfaces
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Flat interfaces, either in their liquid or solid state, are valuable substrates to reconstitute biological membranes and study their structure, molecular composition and ordering under biologically relevant conditions. Interfacial studies have been also carried out under non-equilibrium conditions to look at the variations induced by various biological processes such as protein adsorption and lipid redistribution. However, while many powerful techniques are available nowadays to characterize such systems with high accuracy, access to buried interfaces without inducing perturbations has remained a big challenge.

In this context, the use of neutron reflectometry has been particularly useful to characterize the internal structure of soft thin films with sub-nanometric resolution, without sample damage and for a variety of thermodynamic conditions, giving the possibility of clarifying the interaction mechanisms of biomolecules at interfaces.

This talk gives a brief introduction to the technique and the methods used to reconstitute biomembranes at solid and liquid interfaces. Some example studies will be shown to illustrate how isotopic contrast is unique to map the mass distribution across the interface and to highlight the variation induced by intermolecular interactions reproducing biological functions and processes. Finally, a new setup to study the structure of mutually interacting thin films under controlled nanometric separation distance will be presented, which allows for the first time to explore the full range of structure-force scenarios from macroscopic separation to adhesion-contact in a single reflectometry experiment (Miciuilla et al., Langmuir 2018, 34 (3), 789-800).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Top: schematic representation of a reflectometry study at the air/water interface of a glycolipid monolayer under varying surface area. Bottom: Pressure-area isotherms and volume fraction distribution of components across the interface measured at 15 mN/m at varying subphase composition.}
\end{figure}
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Diels-Alder reaction on functional surfaces: towards the design of smart biosensors

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The potential use of Diels-Alder reaction to design thermoresponsive materials is experiencing growing interest. The applications of that chemistry ranges from remendable polymer networks¹ to the reversible attachment of biomolecules onto surfaces controlled by the surrounding temperature². Our research group already explored the possibility of using interfacial Diels-Alder reaction on functionalized plasma polymer surfaces¹. In the present work, we have evaluated the possibility of applying Diels-Alder chemistry for the controlled immobilization and release of biomolecules to a functionalized plasma polymer surface. More specifically, the goal is to control the attachment of a diene-functionalized biotin, capable of specific conjugation with streptavidin, onto a dienophile (maleimide) rich surface. For this purpose, biotin was first modified with fulvene. Fulvene was selected as the diene because it is expected to undergo retro-Diels-Alder reaction at moderate temperature, which is required to consider any application involving biomolecules. The diene functionalized biotin was analysed via ¹H NMR, ¹³C NMR and mass spectrometry. The functionalization of silicon wafers (used as model substrates) with maleimide moieties was done via plasma polymerization of maleic anhydride followed by a two-step post-functionalization process. Functionalized surfaces were brought in contact with the fulvene-terminated biotin and were characterized via XPS and contact angle measurements. After FITC-streptavidin conjugation with biotin, the samples were analysed via fluorescence microscopy. The observation of fluorescence, analysed against control experiments, indicated that the Diels-Alder between the functionalized surface and biotin occured as expected. Investigations on the reversibility of the system are ongoing. These results are the first steps for the fabrication of smart sensors of biomolecules.

![Figure 1. Principle of reversible immobilization of biomolecules](image)

References
How local changes of matrix curvature can direct collective cell migration through modulation of Erk signaling waves

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Collective migration is a key function of many epithelial tissues, both in physiological (wound healing) and pathological (cancerous metastases) processes. Recent evidence suggests that propagation waves of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase activation determine the direction of the collective cell migration. Interestingly, accumulative evidence shows that single cells respond to cell-scale curvature variations (curvotaxis). However, it remains elusive how local changes of curvature can modulate the propagation of ERK and be integrated to coordinate the collective movement. Here we use a photopolymerization method to form in soft hydrogels well-defined corrugation patterns of different wavelengths, as observed in many native epithelial tissues. Our results show that corrugations induce a uniaxial collective flow of MDCK cells in the direction of the corrugation axis, demonstrating a curvotaxis effect on collective migration. By combining Förster resonance energy transfer (FRET)-based biosensors in MDCK cells with long time-lapse experiments, our findings show that Erk protein activation spreads from cell to cell in a defined dynamic pattern (waves) during collective cell migration on flat hydrogels. We then investigate how the modulation of the local curvature can lead to a mechanical stretch at the single cell level, which can activate ERK through epidermal growth factor receptor (EGFR) activation, and ERK activation triggers cell contraction. The contraction of the activated cell pulls neighboring cells, evoking another round of ERK activation and contraction in the neighbors. Our study raises the question of the critical role of cellular response to external stimuli such as matrix curvature in intercellular signal transduction.
Development of biosensors for the detection of pesticides

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Pesticides are commonly used in agriculture to increase crop yields, but their potential negative impact on human1 and environmental health2 requires to comply with the regulation which sets the maximal pesticide residue concentrations in water intended for human consumption. Gas Chromatography- and Liquid Chromatography-Mass Spectrometry (GC-MS and LC-MS)3,4 are highly efficient techniques for pesticide quantification. However, their use is not suitable for real-time monitoring due to the need for sample pretreatment prior to analysis with sophisticated laboratory based methods. Biosensors, which combine a biorecognition molecule and a transducer, appear as elegant tools to replace conventional methods to eventually enable the detection of analytes in real time, without tedious sample pretreatments. The biorecognition molecule ensures specific interaction with the pesticide, whereas the transducer or detection platform ensures measurement sensitivity.

The present study presents the development of two fluorescence-based biosensors, which employ exonuclease I (Exo I) and aptamer as biomolecules of recognition for the specific detection of glyphosate and thiabendazole, respectively. Glyphosate specifically inhibits the activity of Exo I, whereas the aptamer interacts specifically with thiabendazole. Under optimal conditions, the biosensors enabled the specific detection of both pesticides within linear ranges of 100-500 μM for glyphosate and 1-100 μM for thiabendazole. These biosensors have high potential for rapid, accurate, and specific detection of glyphosate and thiabendazole in environmental and agricultural samples.

![Diagram](image_url)

**Figure 1.** (a) Principle of aptamer/target interaction analysis using exonuclease I followed by fluorescence spectroscopy. (b) The expected percentage of enzymatic digestion in the presence and absence of the target.

References


Investigation of the enzymatic activity of bioactive layer-by-layer assemblies in nanopores using a flow-through microfluidic system

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Currently, significant attention is drawn to the development of novel enzyme immobilization methods to produce competitive biocatalysts for food, pharmaceutical and chemical industries. In this regard, layer by layer (LbL) assembly appears as a particularly interesting method, allowing the deposition under soft conditions of bioactive films with well-controlled thickness, roughness and charge surface by playing on the number of deposited (enzyme/polyelectrolyte) bilayers, deposition conditions and the nature of the polyelectrolyte. In our most recent paper, we showed that highly bioactive branched polyethyleneimine (bPEI)/glucose oxidase (GOx) multilayer films could be assembled within nanoporous polycarbonate membranes (PCm) and, we evaluated the impact of the enzyme confinement on its activity by measurements under static reactional mode (STA). However, for application purposes (e.g. membrane-based bioreactors), evaluating the potential of those assemblies under a dynamic flow through continuous mode (FWT) is of great interest. In this context, the aim of the present work is to evaluate the initial catalytic activity of bPEI/GOx assemblies within PCm under FWT and to understand the impact of the number of bPEI/GOx bilayers (4 or 6) and of polyelectrolyte (PE) concentration (0.15 or 1.0 mg/mL) in this reactional mode. LbL assembly of bPEI/GOx into PCm nanopores were performed as previously described. The enzymatic reaction was carried out in a microfluidic setup consisting of 50 mL falcón tube with a P-CAP where the substrate was placed, a pressure pump connected to a flow rate sensor and a homemade flow through reaction cell. The enzyme activity was measured under a flow rate of 100 µl/min over 1h using the Megazyme GOx activity assay and the immobilization parameters (yield and efficiency) were evaluated. As shown in Figure 1, on one hand, we observe that the assembly of 6 bilayers using 1.0 mg mL⁻¹ of PE for the build-up (sample L6-1) presents the highest activity after 1 h of FWT but, has the lowest efficiency. On the other hand, the assembly of 4 bilayers using 0.15 mg mL⁻¹ of PE for the build-up (sample L4-0.15) presents a similar activity, but a much higher efficiency, as a significantly lower amount of GOx (0.15 mg mL⁻¹) is used for the build-up of the bioactive layer and the activity maintained over 1h, while being the system with the lowest yield of enzyme immobilization. Due its lower cost and time consuming in comparison with the other assemblies, this last system presents very interesting properties for potential applications involving enzymatic reactions under dynamic mode.

Figure 1. Comparative study of enzymatic activity, yield and efficiency of assemblies with 4 and 6 layers (L4 and L6) and containing 0.15 and 1.0 mg/mL of PE under continuous flow through reaction mode.

References
Viscoelastic micropatterned hydrogels for studying cell migration and mechanotransduction

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There is growing evidence that viscoelastic properties of the extracellular matrix (ECM) are a key determinant of cell fate. Indeed, recent studies have shown that substrate’s viscoelasticity mediates important processes, such as cell spreading1, differentiation2 and migration3. However, our current understanding of these complex processes is mainly based on purely elastic matrices, and it is therefore unclear how cells interpret changes of ECM viscoelasticity. In addition, most cellular processes involve a physical restriction imposed by neighbouring cells and the surrounding ECM. For instance, it has been shown that cancer cell migration is directly related to the spatial confinement imposed by the native collagen matrix4. Consequently, viscoelastic cues should be combined with physical confinement to recapitulate the complexity of the cellular microenvironment. Here we developed viscoelastic polyacrylamide hydrogels of constant Young’s modulus (E) but varying viscous properties and combined them with fibronectin micropatterns obtained either with light induced molecular absorption or microcontact printing. Using MCF-10A cells and time lapse microscopy, we employ this platform to study how matrix viscoelasticity mediates cell migration on stiff (E = 3 kPa) and soft (E = 300 Pa) elastic and viscoelastic matrices. Further, we show that this platform can be used to study single cell mechanotransduction in a highly controlled manner, decoupling substrate’s stiffness, viscoelasticity, ligand density and cell shape/spreading.

References


Elaboration of nanoneedles arrays via dry etching, their functionalization and potential applications.

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Silicon nanostructures like nanoneedle arrays present a huge potential for various applications such as photovoltaic cells [1], sensors [2], information storage [3] to name a few. Nanoneedles (NNs) are defined as nanomaterials presenting high aspect ratio. Those belong to two main classes: single needles, externally manipulated to contact cells and tissues (near field microscope (AFM), Micromanipulator) or arrays of vertical high aspect ratio nanostructures supported on a substrate. The former encompasses a wide variety of nanostructures including nanowires, nanopillars, porous nanocones, nanotubes, and nanostraws. Variety of materials/dimensions/shapes make each type of NNs having different properties that befit specific sensing needs, that is to say various applications in mechanobiology, nanoelectrophysiology, optogenetic, nanophotonic, transfection/vectorization (drug delivery) [4]. What we suggest here is the development of innovative and minimally invasive nanoneedle based platforms made of Silicon or Carbon which would present two principal abilities. Firstly, to probe the cells without damaging them while capturing biomarkers of interest. Secondly, to use the NNs as a multimodal detection platform which could provide signal in SALDI-MS, SERS and Electrochemical (EC) sensing methods. As opposed to MALDI-MS, SALDI doesn’t use any organic matrix but is based on an optimized nanostructured surface such as our NNs which could provide better result in terms of S/N ratio. Moreover, the combination of SERS, EC and SALDI-MS already showed in several papers its interest in biosensing to distinguish biomolecules and their toxic isomers [5] or to improve imaging technics based on these methods [6]. To this extent, we present the fabrication techniques of the NN’s. Following the uniform self-assembly of polystyrene particles via nanosphere lithography step, a single step continuous ICP etching was achieved. The interest of this lithography method is to reduce the cost and increase the speed of fabrication of Si NNs compared to common lithography based processes. In parallel, a maskless procedure was used for the fabrication of carbon NNs via a single RIE step. The nanoneedle arrays obtained with both methods were then characterized with water contact angle, AFM, SEM… Then, some of them have been functionalized with organic and inorganic materials and applied for sensing.

References:

Design of biocatalytic detoxification membranes for micropollutants removal from wastewater

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Micro-pollutants have become a worldwide issue of increasing environmental concern, with more and more toxic chemicals entering natural and human ecosystems. Most of these pollutants such as polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) or polychlorinated biphenyls (PCBs) cannot easily be removed from wastewater by conventional water purification methods. Enzymatic catalysis offers a more environmentally-friendly option, on account of its lower energy requirements, moderate operation conditions and non-toxic products. More precisely, laccase is a class of oxidoreductase enzymes with excellent catalytic properties and broad substrate range that meet all the conditions to become a promising option for future water purification. However, the simple use of laccase in solution to deal with these pollutants is not a viable option, due to lack of reusability and enzyme production cost. Their immobilization on a solid substrate therefore represent a very seducing alternative, in order to obtain reusable and highly active biocatalytic materials.

In this work, we investigate the efficiency of the layer-by-layer (LbL) assembly technique to immobilize laccase on nanoporous polycarbonate (PC) membranes. This technique relies on the successive adsorption of oppositely-charged macromolecules through electrostatic interactions in order to form well-controlled multilayer films at the surface of a substrate material. The mild conditions in which the assembly is performed along with the simplicity of the assembly process, makes LbL a very promising immobilization method that preserves enzymatic activity and could easily be scaled up.

The successful immobilization of laccase (harboring a negative net charge at pH higher than 4.0) using branched polyethyleneimine (bPEI) as oppositely charged entity was initially demonstrated on flat surface based on Quartz-Crystal-Microbalance measurements (QCM). After this preliminary step, the immobilization system was used to functionalize PC membranes and assess the relevance of building several [bPEI-Lac] bilayers (BL). It appears that, despite an increased amount of immobilized laccase with 3BL compared to 1BL, the resulting effective activity of the membranes was not drastically improved. This could be explained by a lower substrate accessibility for laccase immobilized in lower layers, or by pore clogging when more BL are deposited. In addition to the initial activity, the reusability of the synthesized membranes was evaluated by performing repeated activity measurements. Unfortunately, it appears that membranes functionalized with a simple LbL method were poorly stable, resulting in a significant drop of activity after the first use. This is explained by the fact that electrostatic interactions, initially responsible for the immobilization of laccase, are not strong enough to form long lasting and stable multilayers, leading to laccase leaching out of the membrane. Glutaraldehyde was therefore proposed as crosslinking agent to try to improve the stability of the formed film. The resulting membranes functionalized with the [bPEI-Lac]-glu system proved to be much more active and reusable than the initial ones.

To conclude, we demonstrated the effective immobilization of laccase using the LbL assembly technique, with bPEI as a counter-polyion. This extremely simple process could be used to functionalize almost any type of solid substrate. We investigated its implementation onto PC nanoporous membranes. It highlighted the necessity of a crosslinking step in order to avoid leaching and obtain reusable materials. As future prospect, we still need to investigate the impact of PC membrane architecture on the resulting material activity. The efficiency of such materials for the removal of actual pollutants should also be assessed, in order to bridge the fundamental knowledge generated so far, to practical applications.
Characterization of bioelectrodes by in situ fluorescence microscopy

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Biological redox systems, such as redox enzymes, can be conveniently characterized by electrochemical techniques. However, since the electrochemical measurement is averaged over the entire surface of the electrode, no data about surface heterogeneities are provided. Therefore, there is a major interest in coupling electrochemical techniques to other methods for collecting simultaneously spatial information [1, 2] and quantifying the surface homogeneity of electro-enzymatic reactivity. Moreover, electrochemical techniques allow to analyze exclusively species confined at the vicinity of the electrode surface, while only indirect clues about phenomena occurring further away from the surface are provided. Precious information about mass transport and reactivity could be obtained by investigating the concentration profiles of the different species near the electrode surface, or in the volume of a porous electrode. In this contribution, we show that in situ coupling electrochemistry with fluorescence confocal laser-scanning microscopy (FCLSM) enables investigation of complex redox systems [3,4]. One of the most interesting features of FCLSM is the possibility to record sets of images with very low depths of field at different coordinates in the axial direction, and thus to reconstruct 3D concentration profiles. Recording fluorescence in the volume adjacent to the electrode under potential control thus enables rebuilding the diffusion layer [3, 4, 5], and to evidence phenomena that occur in the diffusion layer and barely affect the electrochemical response [4]. In situ FCLSM allows to characterize electrochemical reactions involving the direct or indirect generation or the consumption of fluorescent species. We show that the method can be implemented to characterize electro-enzymatic catalysis [5, 6]. For example, enzymatic O2 reduction or H2 oxidation involve proton transfers, which can be evidenced via the fluorescence change of a strongly pH-dependent fluorophore like fluorescein. Local pH changes in the electrode plane can be visualized both during O2 reduction catalyzed by an immobilized bilirubin oxidase and during H2 oxidation catalyzed by an immobilized hydrogenase. Moreover, proton gradients generated during the enzymatic electrode reaction can be readily imaged and their expansion under various experimental conditions can be determined. This paves the way to direct imaging of the evolution of confined environments in porous 3D electrodes during electro(enzymatic) catalysis.

References

Figure 1. Illustration of the experimental setup enabling “in situ” microscopy during the electrochemical reaction.
Surfaces polymères antimicrobiennes

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Il a été montré que des polymères synthétiques possédant une structure amphiphile avec des parties chargées positivement et des parties hydrophobes, présentent des propriétés antibactériennes puissantes1. Parmi ceux-ci, les polyanônes (PI) sont particulièrement intéressants puisqu’ils bénéficient d’une balance charge/hydrophobicité modulable et d’une charge présente sur le squelette (et non en groupe pendant). Des travaux récents ont aussi montré que ce type de polymère en solution n’induit pas de résistance bactérienne.2

Depuis plusieurs années, le NIMBE/CEA travaille sur les polyanônes (PI) et plus particulièrement sur leur immobilisation sur diverses surfaces natives (verre, polyéthylène, etc.)3. Des méthodes par greffage chimique, impression 2D ou formation de mélanges-maîtres ont ainsi été mises au point. Les résultats issus de l’étude microbiologique sur différentes souches ont montré que nos surfaces greffées PI étaient particulièrement efficaces pour inhiber la croissance des bactéries (effet bactériostatique), tout en les piégeant (effet pro-adhésif). Nous avons également montré que cet effet bactériostatique est plus important lorsque les segments aliphatiques des PI sont plus longs. L’effet antibactérien de ces surfaces modifiées est également souche-dépendant. Ce type de greffage n’a jamais été décrit auparavant et a été caractérisé de manière exhaustive, afin de démontrer sa robustesse et notamment l’absence de relargage d’espèces chimiques.

Ces surfaces modifiées et modulables sont particulièrement intéressantes pour la décontamination et offrent des perspectives dans les domaines de la santé, la défense et plus largement, dans le domaine industriel.

References
Fonctionnalisation de surface pour la détection bactérienne et apport de la QCM-d pour le développement d’un biocepteur basé sur les interféromètres de Mach-Zehnder

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La crise sanitaire mondiale de ces dernières années, et celles à venir, confirment le besoin d’outils d’analyses performants et rapides de notre environnement. Les capteurs biologiques (biocepteurs) sont des outils phares pour cette application. Les dernières avancées technologiques pour le développement des micro et nano-systèmes permettent d’espérer l’apparition de biocepteurs plus performants et susceptibles d’être intégrés dans des systèmes fluidiques pour produire des appareils commerciaux d’analyse rapides et fiables. Le projet CARNOT NEO (New Exposome Observer) conduit au CEA-Leti s’inscrit dans cette démarche et s’appuie sur le développement de transducteurs en silicium de différentes natures, des compétences en fonctionnalisation des surfaces et en intégration sur systèmes fluidiques.

Néanmoins, la transformation de ces transducteurs en biocepteur nécessite d’effectuer une fonctionnalisation de surface localisée afin de fixer des sondes biologiques correspondant aux cibles d’intérêt¹. Le projet NEO s’intéresse à l’analyse de la qualité de l’eau et notamment aux contaminations bactériennes de type Escherichia coli et Entérococques. Les sondes biologiques choisies sont des anticorps polyclonaux produits contre différents antigènes de ces espèces.

La microbalance à quartz avec dissipation (QCM-d) est utilisée comme un modèle des transducteurs à fonctionnaliser. Cette technique permet de tester différentes méthodes de fonctionnalisation d’immobilisation des sondes biologiques sur les quartz QCM avant de travailler sur les transducteurs finaux².³. Le point de fonctionnement retenu fait ensuite l’objet d’un transfert de la méthode de fonctionnalisation depuis le quartz fonctionnalisé en flux, au transducteur fonctionnalisé par chimie localisée. Le transducteur présenté dans ce poster fonctionne à partir d’interféromètres de Mach-Zehnder (MZI) produits sur un substrat silicium et miniaturisés de telle façon à pouvoir obtenir plusieurs dizaines d’interféromètres sur un centimètre carré. La fonctionnalisation localisée de ces MZI permettra alors le mutiplexage de l’analyse bactérienne.

References
Bio-inspired multi-enzymatic surfaces for antibiofilm protection

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Surface protection strategies against biofilms remain challenging as protection efficacy tends to decrease promptly over time. Current approaches rely on the two following principles: antiadhesion to prevent microbial colonization; and antibacterial effect to kill attached cells. Existing antiadhesion strategies have specific efficacy targeted to certain species, certain bacterial adhesive processes or certain media and thus have a limited range of action. This limitation can lead to biofouling over time reducing the antiadhesion efficacy even more. Due to their “one-shot” nature, some antimicrobial surface treatments can also have limited long-term action. However, antimicrobial enzymes have already been considered as an interesting alternative since their activity is “re-usable” as long as the catalytic site is active [1]. In the present work, we focused on a bio-inspired multi-enzymatic system, a supramolecular nanomachine made of several enzymes anchored on a protein scaffold. Our system was made of a protein with several receptors sites and which serves as a skeleton onto which antibacterial enzymes were attached, namely, lysozyme and lysostaphin that both degrade peptidoglycans in bacterial cell wall. DNase, an enzyme that cleaves extracellular DNA was also docked on the system as an “antiadhesion” and “antifouling” agent. Enzyme anchoring was achieved by the use of interactions between ligands on the enzymes and receptors on the scaffold. Atomic force microscopy (AFM)-based single-molecule force spectroscopy was performed with tips functionalized with enzymes of interests and showed these interactions were highly specific and reversible. Enzyme proportions and relative positions on the scaffold were achievable because of the high specificity of ligands-receptors interactions. Micrococcus luteus (Gram +), Staphylococcus aureus (Gram +), and Escherichia coli (Gram -) viability was showed to be reduced in presence of the enzyme-scaffold system thus revealing its antimicrobial effect. The antimicrobial effects were lessened when enzymes were grafted without a protein scaffold which demonstrates that the scaffold improved the orientation and spatial conformation of enzymes and led to an optimized antimicrobial activity. The presented bio-inspired system combined antimicrobial enzymes as well as antiadhesion agents capable of killing Gram positive and Gram negative bacteria. The reversibility of ligands-receptors links used to dock the enzymes gave promising insights into renewing enzymes without grafting a new scaffold and thus insuring sustainable antimicrobial effects over time.

Principle of the bio-inspired antibiofilm strategy, AFM-based measurement to demonstrate the specific ligand-receptor interaction and antimicrobial efficiency of functionalized surfaces.

References
The extracellular matrix stiffness promotes the invasiveness of breast cancer epithelial cells

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Tumor progression alters the composition and physical properties of the extracellular matrix (ECM). Particularly, increased matrix stiffness has profound effects on tumor growth and metastasis in breast tissues. While one of the major contributing factors is increased density of collagen fibers in the ECM, the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells remain unclear. Here we used Gelatin hydrogels (GelMA) derived from native type I collagen through partial hydrolysis and functionalized with methacrylate groups to reproduce in vitro the main physico-chemical properties of breast tissues. We used the Irgacure 2959 photoinitiator to control the polymerization of GelMA hydrogels through UV illumination. Our findings show that the rigidity of the hydrogels increases from 2 kPa (soft) to 15 kPa (stiff) by doubling the gelatin concentration, which allows to mimic the rigidity of healthy and tumoral breast tissues. Normal (MCF-10A) and tumoral (MDA-MB-231) epithelial cells were cultured on soft and stiff GelMA to investigate the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells.
Development of an Artificial Bioresorbable Ligament Based on pNaSS Grafted PCL

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The anterior cruciate ligament (ACL), known to stabilize the knee movement, is one of the most frequently injured structure during sport activities. Autograft and allografts are considered as the current methods for surgical intervention, however, these strategies represent many drawbacks such as donor site morbidity and risk of disease transmission\textsuperscript{1}. Therefore, the development of a synthetic scaffold able to maintain the main function of the damaged ACL and consequently to regenerate the native ACL synthesis is the alternative approach. PCL as scaffold material is widely used in various biomedical applications as tissue engineering due to the appropriate properties such as biocompatibility, biodegradability, high mechanical strength and surface hydrophobicity that affect the cells interaction. In the present study, PolyCaprolactone (PCL) has been chosen to develop biodegradable synthetic ligaments mimicking the desired properties of ACL\textsuperscript{2}. The PCL surface was functionalized with poly (Sodium Styrene Sulfonate) (pNaSS), characterized by the presence of hydrophilic sulfonate group having an impact on cells behavior as cells adhesion, proliferation and differentiation\textsuperscript{3}. pNaSS is as well characterized by the low toxicity and the high thermal stability\textsuperscript{4}. A thermal grafting method was processed to functionalize the PCL surface with pNaSS. The colorimetric assay (Toluidine Blue) has indicated the success of pNaSS grafting. Moreover, it has been demonstrated that the pNaSS grafted onto the PCL ligament surface did not alter the physico-chemical properties of PCL. The grafted pNaSS enhance also the cellular response by increasing the affinity of Fibroblast cells adhesion and proliferation comparing to ungrafted PCL ligaments.

References
Dynamics of transient cell cruising in confined microenvironments

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The migration of epithelial cells through dense tissues and tight spaces is a crucial process in tissue development, homeostasis, and diseases such as cancer. However, how spatial confinement affects cell migration dynamics is still not well understood. We investigated the transient migration events of epithelial cells on adhesive dumbbell-shaped micropatterns that lead to repeated back and forth migration events. By tuning the dimensions of the central narrow bridge that connect two squared-shape adhesive sites, we show that the spatial confinement imposed by the bridge geometry influences the migration velocity. Our findings show that imposing narrower bridges increases the cell migration speed through large cellular extensions. Interestingly, extending the length of the narrower bridges increases significantly the success rate of crossing up to 95%. We show that the crossing rate and the dynamics of transient migration are both controlled by a morphological switch imposed by the bridge aspect ratio. Indeed, epithelial cells on longer bridges switch from an extended and slow morphology to a fast and compacted phenotype with a steady polarization state, raising the question of the existence of a polarization memory in confined cells. To address this question, we investigated the role of the actin cytoskeleton and characterized the expression of epithelial to mesenchymal markers in these two opposite phenotypes.
Surface colonization by microorganisms is equally of concern for human and environmental health and for the energy sector with tremendous associated economic losses. If hospital acquired (nosocomial) infections are health threatening, microbially induced corrosion (MIC) leads to medical devices and to industrial setting failures in both health and energy sectors. There is thus an urgent need to find biocide-, antibiotic- and nanoparticle- free solutions to combat MIC, i.e. biocorrosion. To counteract this deleterious process, we recently initiated investigations using natural, antimicrobial chitosan coatings on metal surfaces.

We combined adhesion tests, profilometry, electrochemical characterization and X-ray photoelectron spectroscopy (XPS) prior and subsequent to incubation with *desulfovibrio vulgaris*. This bacterium, in anaerobic conditions, reduces sulphate, which produces corrosive hydrogen sulfide. The mains outcomes of these investigations demonstrate the preparation by dip coating of adherent films of thickness in the μm range, which are stable even after five weeks of incubation with bacteria and which prevent both corrosion and biocorrosion.
La plateforme Fonctionnalisation de Surface du CEA-Leti : une expertise au service des microtechnologies pour la santé

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Pionnier dans le domaine des micro et nano-technologies, le CEA-Leti est un institut de recherche technologique développant des solutions applicatives innovantes, facteurs de compétitivité, et répondant aux défis mondiaux actuels, notamment les énergies propres et sûres, la santé et le bien-être, le transport durable et les technologies de l’information.

Depuis le début des années 2000, au sein de sa plateforme PRISM du CEA-Leti, notre équipe développe un catalogue de procédés de fonctionnalisation chimique et de greffage de biomolécules s’intégrant à la fabrication de micro et nanosystèmes et répondant au maximum aux critères de la « chimie verte ». Avec plus de 170 m² de salle blanche de classe ISO6 équipée de pièces en lumière inactinique, la plateforme permet de travailler d’après les normes de propreté imposées par les micro et nanotechnologies.

Plusieurs équipements y sont disponibles permettant de faire des dépôts en phase gaz (1), en milieu supercritique (2,3), par dispense de micro-goutte (3), sous micro-onde, à la tournerette, par spray et par électrofiliation. Des équipements de caractérisation de surface sont également disponibles pour la mesure du potentiel zéta, la mesure de tension de surface et d’angle de contact, la mesure de fluorescence, la mesure de rugosité de surface par AFM et la mesure de porosité et de surface spécifique, en plus des équipements de la plateforme NanoCaractérisation du CEA Grenoble (XPS, MEB, AFM, IR...).

Récemment, de nouveaux équipements nous permettent d’élargir la gamme de dépôts vers des couches plus épaisses et de développer des procédés de fonctionnalisations de surface par polymérisation plasma ou par iCVD pour l’antibiofouling (5).

Grâce à ces équipements de dépôt et de caractérisation de surface à l’état de l’art, une « boîte à outils » axée sur la création d’une interface chimique, conçue pour des systèmes intégrés en tenant compte des contraintes de fabrication et d’application a ainsi été mise au point.

References
Mechanoresponse of epithelial monolayers to in-plane and out-of plane curvatures imposed by 3D microwells

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The organization of epithelial tissues with precise spatial definition is essential to various biological processes and to generate curved epithelial structures, such as lobules in breast, lung and kidney tissues. However, the regulation of the architecture and dynamics of collective epithelial assemblies by the matrix curvature remains unclear. To address this challenge, we designed a new photopolymerization method through an optical photomask to create microwells of various diameters in hydrogels that mimic the native organization of epithelial monolayers in soft lobular structures. Using these well-defined microwells of different aspect ratios, we decoupled how in-plane and out-of plane curvatures modulate the mechanoresponse of epithelial tissues. Our findings show that in-plane curvature leads to the formation of an actomyosin supracellular structure formed at the edge of the microwell, while convex out-of plane curvature imposed at the microwell entrance leads to a vertical orientation of the nuclei towards the microwell axis. We demonstrated that increasing the out-of plane curvature leads to more flatten and elongated nuclear morphologies with high levels of chromatin compaction. Interestingly, our results show that epithelial cells exhibit higher directionality and speed around the microwell edge, demonstrating that the out-of-plane curvature significantly enhances the cellular trafficking, referred as curvotaxis. These findings demonstrate the importance of in-plane and out-of plane curvatures in epithelial organization and how both can be leveraged to facilitate the engineering of curved structures to study curvature-dependent mechanotransduction pathways.
DEVELOPMENT OF A MACRO-POROUS PNIPAM BASED ACTUATOR FOR BRAILLE DEVICES

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The bottleneck of the current haptic technology for a low cost and efficient Braille device lies in the actuation method. A simple and low-cost actuation system with high durability is crucial for affordable devices. Thus, polymeric soft actuators present high potential to fill such void in the haptic technology. Here, we demonstrate the state-of-the-art system for inclusion of a soft actuator into the haptic technology. This paper reports the development of a soft actuator based on thermosensitive PNIPAM gel for a smart Braille device application. For the first time, a hydrogel actuator is optimized as a fast actuator for a Braille device. This material combines high robustness, large swelling amplitude and fast actuation speed. To achieve this goal, we synthesize hydrogel with a macro-porous structure obtained from a sacrificial template. With the introduction of macro-porosity to the organically reticulated (OR) gels, we were able to decrease the shrinking and re-swelling durations from hours to 15s and 20s, respectively. The acquired highly porous (HP-OR) gels with 60% porosity were also tested mechanically and proved to fully preserve its elastic modulus even after 100 compression cycles. Moreover, a novel single pin Braille device is developed. Nichrome wires and Peltier devices are utilized for heating and cooling, respectively. The pin displacement upon heating and cooling is measured to be ~1.5 mm when no force is exerted. A displacement of ~0.5 mm when 5.0 g of force is continuously kept on the pin tip throughout retraction and ascension is observed. Finally, the active force generated by the Braille tip is also tested. Results revealed that 0.9 g of force can be generated by the HP-OR gel actuator and preserved over 10 retraction-ascension cycles [1].

![Figure 1](image.png)

**Figure 1:** (a) Schematic of the single pin Braille system and the photograph of the setup.

References

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